Effects of bisphenol A on early development of the ascidian *Phallusia mammillata* (Chordata, Tunicata)

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Bisphenol A (BPA) is an organic compound used in the manufacture of polycarbonate plastic and epoxy resins that is released into the environment from sewage treatment effluent, landfill leachate and degradation of plastic products. BPA can act both as a teratogenic substance and as an endocrine disruptor. The phylogenetic position of tunicates as sister group of vertebrates and their cosmopolitan distribution in marine ecosystems coupled with their ecology and easy manipulability make them reliable model organisms for ecotoxicology bioassays. Here we evaluated the effects of different concentration of BPA (0.1, 0.5, 1, 5, 10, and 20 µM) on ontogenetic processes sensitive to environmental pollutants in the ascidian *Phallusia mammillata*. To test the effects of this substance on sperm viability we fertilized eggs with sperm pre-exposed for 30 minutes to BPA. One hour post fertilization (hpf) we calculated the percentage of eggs that reached the 2-cell stage compared to control. Then we analyzed the consequences of a fertilization performed directly in BPA solutions, counting the percentage of 2 cell-stage embryos 1 hpf. To test the effects on embryogenesis, we analyzed hatched larvae developed from 2-cell embryos exposed to BPA. We evaluated the number and type of malformations under a dissection microscope and we performed immunostaining of central nervous system (CNS). Exposure of sperm to BPA did not influence fertilization rate. Co-exposure of eggs and sperm at concentrations higher than 5 µM caused incomplete division of eggs, producing heart shaped embryos that did not develop further. Regarding embryogenesis, 10 µM BPA caused specific malformations at central nervous system, with an increased distance between otolith and ocellus, or an extrusion of ocellus from the sensory vesicle. Embryonic development was significantly altered at 20 µM concentration. 97% of the larvae presented a severely affected phenotype with short and kinked tail. Immunostainig with anti β-tubulin antibody showed an altered pattern of fibers in CNS. These results showed that the most sensitive process to BPA is the first cell division. When the 2-cell stage embryos are treated, higher concentration are required to alter the correct development of CNS. BPA confirmed its teratogenic effects on ascidians and its interference with CNS development even if the mechanism of action in this group is still to be clarified.